DIVISION OF DIGESTIVE DISEASES AND NUTRITION

FY 1999 Program Plan

RESEARCH PROGRESS REVIEWS

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DIVISION OF DIGESTIVE DISEASES AND NUTRITION

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Research Progress Reviews

LIVER DISEASES PROGRAM

I. TITLE: Pediatric Liver Disease and Genetics: Further characterization of the major pediatric forms of liver disease-Progressive familial intrahepatic cholestasis (PFIC) and benign recurrent intrahepatic cholestasis (BRIC)

BACKGROUND: Bile formation is an osmotic secretory process of the liver that is driven by the active concentration of bile salts and other biliary constituents in the bile canaliculi. The transport of solutes from the blood to the bile is driven by two transport systems, one in the plasma membrane of the basolateral (sinusoidal) surface and one on the apical (canalicular) surface of the hepatocyte. The sinusoidal systems for bile-salt uptake in hepatocytes include a sodium-taurocholate cotransporter (NTCP) and a sodium-independent organic-anion transporter (OATP). The canalicular membrane contains several ATP-dependent export pumps: the multidrug-resistance gene or P1-glycoprotein (MDR1); the phospholipid transporter mulitdrug-resistance –P3-glycoprotein (MDR3); the multi-organic-anion transporter (MRP2 or cMOAT); and the bile-salt-export pump (BSEP or SPGP). Recent studies have identified the genes coding for these transporter systems allowing for an analysis of the role of these transporter systems in inherited cholestatic disorders.

RECENT FINDINGS: Molecular changes have been identified in patients with cholestatic disorders related to the genes of the hepatocellular transport systems. Decreased or even absent expression of specific hepatocellular transport proteins have been found in several clinical forms of cholestasis. Progressive familiar intrahepatic cholestasis (PFIC) is a severe type of cholestatic liver disease that is inherited as an autosomal recessive trait. The disease presents in infancy and results in liver failure. Three types of PFIC are now recognized with differing molecular defects: Type I (Byler's disease) has been mapped by positional cloning to chromosome 18q21-22 with the molecular defect a mutation in P-type ATPase (FIC1); Type 2 (found in Middle East populations and Greenland and Sweden) results from a mutation in SPGP gene on chromosome 2q24; Type 3 results from a mutation in MDR3 on chromosome 7q21. Benign recurrent intrahepatic cholestasis (BRIC) is characterized by recurrent episodes of intraheptic cholestasis lasting days to months and that can resolve spontaneously without lasting liver damage. The mutation has been mapped to the 18q21-22 locus with a defect in the P-type ATPase (FIC1).

SIGNIFICANCE: The molecular abnormalities explain the impairment of transport functions, with a subsequent reduction in bile flow and the development of cholestasis.

FUTURE DIRECTIONS: Hepatocytes transporter defects have not been identified in the MDR1 transporter or in sinusoidal transporters and related to cholestasis. In addition, other pediatric liver diseases that develop neonatal cholestasis, such as biliary atresia, need to be reevaluated for the known gene mutations that cause cholestasis.

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R01DK25636	Boyer , J. L.	Yale Univ School of Med
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Trauner M, Meirer PJ, Boyer JL. "Molecular Pathogenesis of Cholestasis." N. Engl J. Med 1998; 339:1217-27.

Bull LN, van Eijk MJT, Pawlikowska L et al. "A Gene Encoding a P-type ATP-ase Mutated in two forms of Hereditary Cholestasis." Nature Genet 1998;18: 219-24.

II. TITLE: Liver Gene Therapy: New Approaches to Altering Abnormal Genes

BACKGROUND: Human gene therapy has been directed at the genetic diseases of the liver. To date, limited clinical efficacy has been shown. Major problems exist with regard to the generation of the appropriate vector to be utilized, long-term expression of the transgene and regulation of transgene expression within a therapeutic concentration range. Thus, new and novel methods for genetic -based therapeutics need to be developed. One such novel approach is based on the premise that single-base (point) mutations within a particular gene are the cause of many of the known inherited liver diseases. This is true for α-1 antitrypsin deficiency or hemophilia where the mutation results in either the production of a nonfunctional mutant protein or the entire loss of the protein product. For the case of a mutation is a single gene, the most desirable form of gene therapy would be targeted homologous recombination between the mutated gene and a new normal version of the gene. The frequency of this form of therapy is very low and is subject to random insertion into the genome of the correct version of the gene. Thus, a novel form of

therapy is the formation of RNA/DNA hybrid (chimeric molecules) at the site of the mutation. These molecules are highly active in homologous pairing reactions resulting in correction of the mutation in vivo.

RECENT FINDINGS: A chimeric RNA/DNA oligonucleotide was constructed to induce sequence mutation in the rat factor IX gene. Oligonucleotides were targeted to hepatocytes in cell culture or in vivo by intravenous injection. Nucleotide conversion was both site-specific and dose-dependent. The mutated gene was associated in vivo with significantly reduced factor IX coagulation activity.

SIGNIFICANCE: The results of this study demonstrate that single base-pair alterations can be introduced in hepatocytes in situ by RNA/DNA oligonucleotides suggesting a potentially powerful strategy for hepatocyte gene repair without the use of viral vectors. The study also showed that both isolated hepatocytes and intact liver are amenable to targeted genomic nucleotide conversion. This methodology may have enormous implications for the future of gene therapy and allow for correction of many serious genetic diseases.

FUTURE DIRECTIONS: Future studies need to address the reproducibility and stability of the nucleotide conversions. Ultimately this method needs to be attempted in a genetic liver disease of humans such as α-1 antitrypsin deficiency or hemophilia.

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R01DK44649	Steer, C. J.	Univ Minnesota Med sch

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Kren BT, Bandyopadhyay P, Steer CJ. “In vivo site-directed Mutagenesis of the Factor IX gene by Chimeric RNA/DNA Oligonucleotides.” Nature Medicine 1998; 4:285-90.

III. TITLE: Liver Transplantation and End Stage Liver Disease

BACKGROUND: Mortality from end-stage liver disease declined yearly for over 20-years, but in the last 2-years has begun to increase killing more than 25,000 Americans in 1997. Liver transplantation is the only effective treatment for end stage liver disease with more than 4,000 liver transplantations now performed annually in the United States. Because of a shortage of donor livers, it is currently not feasible to increase further the number of transplantations. Thus, there is acute interest in optimal allocation of this scarce resource. One approach, as shown below, is to determine optimal timing of transplantation with the goal that patients would receive transplantation when needed but before they become so sick that the risk of death and graft loss becomes unacceptably large. Large databases have also begun to evaluate predictive factors of survival for chronic hepatitis C, a major reason for liver transplantation.

RECENT FINDINGS: In 1989 the efficacy of liver transplantation in primary biliary cirrhosis (PBC) was demonstrated by showing that actual patient survival following transplantation was significantly better than without transplantation as predicted by a mathematical survival model ("Mayo natural history model"). Using this model and outcomes following transplantation, the optimal time to perform liver transplantation in PBC has been determined. A risk score is constructed using age, bilirubin, albumin, prothrombin time, and the presence or absence of edema. The risk of death following transplantation remains low until reaching a risk score of 7.8. In contrast, risk scores greater than 7.8 are associated with a progressively increased mortality. Resource utilization measured by the days in the intensive care unit and hospital and the requirement for intraoperative blood transfusions is significantly greater in recipients who have higher risk scores before transplantation.

This model has been adapted to a web site that allows easy calculation of expected natural history without transplantation in PBC and in primary sclerosing cholangitis, another major cause of end stage liver disease. This site also allows calculation of predicted blood usage, length of stay in ICU, and occurrence of significant complications in patients undergoing liver transplantation for these diseases.

Analysis of a large cohort of patients transplanted for chronic hepatitis C in the Liver Transplantation Database has shown that the pre-transplantation level of hepatitis C viral RNA in serum was the sole predictive factor for decreased survival in this cohort of patients. Indeed, in patients with low levels of HCV RNA in serum, survival after liver transplantation is excellent, and as good as survival after transplantation for cholestatic liver diseases, such as PBC. In contrast, among patients with high levels of HCV RNA in serum before transplant, survival is poor and a major cause of mortality is recurrence of hepatitis C.

SIGNIFICANCE: Clinicians can now easily use this natural history model to make management decisions for their patients with chronic cholestatic liver diseases. Furthermore, studies can now be undertaken to evaluate therapy of hepatitis C aimed at patients with the highest risk for complications, i.e. those with high initial levels of HCV RNA. Such work demonstrates the successful translation of NIH funded research into clinical practice.

FUTURE DIRECTIONS: This successful modeling approach is now being adapted to hepatitis C, which has become the most common reason for liver transplantation. Studies of pre-emptive therapy of hepatitis C and of therapy of recurrent disease are now being designed.

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Kim, WR, Wiesner, RH, Therneau, TM, Poterucha, JJ, Porayko, MK, Evans, RW, Klintmalm, GB, Crippin,JS, Krom, RA, Dickson, ER. "Optimal Timing of Liver Transplantation for Primary Biliary Cirrhosis," Hepatology 1998;28:33-38.
Charlton M, Seaberg E, Wiesner R, Everhart J, Zetterman R, Lake J, Detre K, Hoofnagle JH. "Predictors of Patient and Graft Survival Following Liver Transplantation for Hepatitis C." Hepatology 1998;28:823-830.

Mathematical Models in Cholestatic Liver Disease at:
http://www.mayo.edu/int-med/gi/model/mayomodl.htm

IV. TITLE: Chronic Hepatitis B and C: Further Advances in Therapy

BACKGROUND: Hepatitis B and C are the two most important causes of acute and chronic hepatitis in the United States and account for approximately one-third of liver transplantations done in adults in the United States. Therapy of hepatitis B has progressed slowly since the initial studies of alpha interferon conducted at the Clinical Center of the NIH in the early 1980's. More recently, oral nucleoside analogue drugs have been developed for therapy of hepatitis B and are now undergoing clinical evaluation. The hepatitis C virus was discovered in 1989, yet even before the discovery of the virus and development of diagnostic tests, several advances had been made in therapy of this disease. The first report of successful use of alpha interferon in chronic hepatitis C was from the Clinical Center of the NIH where NIDDK investigators showed that a prolonged course of treatment led to improvement in the liver disease and fall of liver enzymes into the normal range. Once the virus was discovered, it was shown that these patients had hepatitis C and that therapy led to disappearance of HCV RNA from the serum. Based upon these preliminary results, controlled trials were carried out using various doses and regimens of alpha interferon, which led to the approval of interferon for treatment of hepatitis C in 1991. Nevertheless, therapy was still problematic: less than half of patients responded to therapy and many relapsed when treatment was stopped.

RECENT FINDINGS: In the last year, alpha interferon was approved for use in children with hepatitis B, the result of a multi-national study in which members of the Liver Diseases Section, NIDDK took part both in the design and conduct of the trial. For hepatitis C, several large, multicenter trials have shown that the response to alpha interferon can be increased two to three fold by the addition of ribavirin, an oral nucleoside analogue. Thus, a twelve month course of the combination of alpha interferon and ribavirin led to sustained eradication of viremia in 40 percent of patients. This combination was first used in the United States by intramural scientists in NIDDK who subsequently helped in the design of the study, but did not actually participate in the final trial. The results of these studies led to the FDA approval of this combination therapy for hepatitis C in December 1998.

At issue, however, is whether the responses to alpha interferon are sustained and whether treatment truly results in permanent eradication of virus infection and resolution of the disease. In long term follow-up studies by members of the Liver Diseases Section, NIDDK, patients with hepatitis C who had a sustained response to therapy in 1984-1986 were evaluated carefully 10 to 12- years after treatment. All responders were found to be without evidence of disease and without detectable HCV RNA in either blood or liver. Thus, sustained responses to treatment most likely represent a eradication of virus infection and "cure" of the disease. Hepatitis C is the first chronic viral infection found to be curable by antiviral therapy.

SIGNIFICANCE: Hepatitis C affects 1-2 percent of the United States population, probably 4 million adults. This disease results in cirrhosis in approximately 20 percent of infected individuals and now ranks as perhaps the major cause of cirrhosis and end-stage liver disease in the United States. A safe and effective therapy of hepatitis C would decrease the mortality and morbidity of liver disease considerably. With current therapies, 30-40 percent of patients might be cured of this chronic viral infection and liver disease.

FUTURE DIRECTIONS: Improvements in therapy of hepatitis B and C are needed, as well as translational efforts to inform the public and the medical care profession about the importance of hepatitis C and means of prevention and treatment.

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Lau DYT, Kleiner DE, Ghany MG, Park Y, Schmid P, Hoofnagle JH. "Ten-year Follow up After Interferon-α therapy for Chronic Hepatitis C." Hepatology 1998;28:1121-1127.

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Sokal EM, Conjeevaram HS, Roberts EA, et al. "Interferon alfa therapy for Chronic Hepatitis B in Children: a Multinational Randomized Controlled Trial." Gastroenterology 1998;114:988-995.

V. TITLE: Liver Cell Development and Use in Treatment of Genetic Liver Diseases: Identification of a New

Animal Model of Liver Cell Transplantation and Repopulation and the Use of Liver Cell Transplantation To Treat Genetic Liver Disease

BACKGROUND: Repopulation of a chronically diseased liver via hepatocyte transplantation would represent a valuable alternative to whole organ transplantation. Major problems in hepatocyte transplantation studies have been the limited growth of transplanted cells in the recipient organ as well as the identification of pleuripotent stem cells. The identification of such liver stem cells would represent a major advance in the field by providing a population of cells with a large growth potential coupled with a cellular differentiation capacity to perform the complex biological functions of the liver. In addition, such cells may have a selective growth advantage over endogenous hepatocytes a property needed to repopulate a dysfunctional organ.

RECENT FINDINGS: A new animal model has been developed which allows for hepatocyte repopulation through the selective proliferation of transplanted cells. The selective growth advantage is derived using a new strategy that interferes with the proliferative capacity of resident hepatocytes followed by transplantation of normal hepatocytes in conjunction with partial hepatectomy. This strategy allows for the tracking of genetically marked transplanted hepatocytes thereby elucidating the proliferation, expansion and integration of the transplanted hepatocytes into the hepatic parenchymal structure of the liver. The current studies have noted near total replacement 98-99 percent of hepatic mass by hepatocyte transplantation for up to nine months with normal liver function. In other studies, hepatocyte transplantation was reportedly used for the first time in a patient with Crigler-Najjar Syndrome Type I. Crigler-Najjar Syndrome Type I is a recessively inherited disorder characterized by severe unconjugated hyperbilirubinemia beginning at birth. Hepatocyte transplantation represents an alternative to whole organ transplantation because hepatic architecture and function (except for the hyperbilirubinemia) is normal in this syndrome. The present study reports that allogeneic hepatocytes were safely infused through the portal vein, survived for more than eleven months and partially corrected the metabolic disorder in the patient.

SIGNIFICANCE: The development of a new animal model for liver repopulation will allow for a more detailed analysis of liver cell transplantation and the characterization of liver stem cells for use in transplantation studies. The report of the initial use of liver cell transplantation for the therapy of genetic liver disease indicates the feasibility of the approach in a clinical setting.

FUTURE DIRECTIONS: Future studies need to identify liver stem cells and characterize their use in animal models of liver cell transplantation and

repopulation. In addition, numerous biological properties of the cells need to be identified in vivo, most importantly their function, growth and longevity.

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Laconi E, Oren R, Mukhopadhyay DK et al. “Long Term, near Total Liver Replacement by Transplantation of Isolated Hepatocytes.” Am J Pathol 1998;153:319-29.

Fox IR, Roy Chowdhury J, Kaufman et al. “Treatment of Crigler-Najjar Syndrome Type I with Hepatocyte Transplantation. “ N .Engl. J Med. 1998;338:1422-26.

PANCREAS PROGRAM

VI. TITLE: Acute and Chronic Pancreatitis: Identification of Tumor Necrosis Factor-alpha (Tnf-α) Production by Pancreatic Acinar Cells and the Presence of the Cystic Fibrosis Transmembrane Receptor (Cftr) in Pancreatic Acinar and Ductal Cells- Potential Roles in Pancreatitis

BACKGROUND: Acute and chronic pancreatitis are major health care problems in the United States. The pathological mechanisms that cause acute pancreatitis and perpetuate chronic pancreatitis remain to be elucidated but are related to pancreatic secretion. Alternatively, there appears to be a genetic predisposition or susceptibility to pancreatitis through the presence or mutation of specific genes. Most recently, mutations have been identified in the cationic trypsinogen gene which leads to hereditary pancreatitis. Thus, the genetic basis for the inherited form of pancreatitis has been identified.

RECENT FINDINGS: In a study using an animal model of pancreatitis,

pancreatic acinar cells were shown to produce, release and respond to TNF-α. TNF-α is a major mediator of the acute inflammatory response and stimulates cell death through receptor mediated events leading to either apoptosis or necrosis. In this study, TNF-α messenger RNA as well as the mediator (TNF-α) were present in the pancreas of animals with experimentally-induced pancreatitis. In addition, pancreatic acinar cells expressed the cellular receptors for TNF-α. On receptor activation, pancreatic acinar cells were shown to have activated transcription factors and subsequently undergo cell death via apoptosis.

In other studies, experiments using immunological and functional reagents showed, for the first time, the expression of the CFTR on pancreatic acinar and ductal cells. These studies indicate that potential mutations in the CFTR could lead to modified secretion of digestive enzymes in the pancreas.

SIGNIFICANCE: More than 800 mutations in the CFTR have been identified and attempts have been made to elucidate relationships between CFTR genotype and disease phenotype. Between 6-37 percent of individuals with idiopathic pancreatitis have a CFTR mutation on at least one chromosome. The present study provides a potential link between genotype and disease phenotype by providing evidence that a mutation in the CFTR could modify both acinar and ductal enzyme secretion thus leading to pancreatitis. Additionally, acinar cells have now been shown to have cellular receptors for TNF-α thereby defining a pathogenic mechanism for pancreatic cellular apoptosis seen in pancreatitis.

FUTURE DIRECTIONS: Further studies are needed to define the molecular and genetic mechanism of acute and chronic pancreatitis. Molecular regulation studies are needed to define gene expression requirements in the induction of pancreatitis and new animal models of pancreatitis using gene knock-out techniques would facilitate this understanding. Furthermore, future studies modulating expression of mediator(s) of inflammation could potentially identify targets to reduce the inflammatory response in the pancreas.

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Zeng W, Lee MG, Yan M et al. “Immuno and Functional Characterization of CFTR in Submandibular and Pancreatic Acinar and Ductal Cells.” Am J. Physiol 1997;42:C442-55.

Freedman, SD. “New Concepts in Understanding the Pathophysiology of Chronic Pancreatitis.” Int. J. Pancreatology1998;24: 1-8.

Gukovskaya AS, Gutovsky I, Zaninovic V et al. “Pancreatic Acinar Cells Produce, Release and Respond to Tumor Necrosis Factor-α. Role in Regulating Cell Death and Pancreatitis.” J. Clin Invest. 1997;100:1853-62.

GASTROENTEROLOGY PROGRAMS

VII. TITLE: Inflammatory Bowel Disease

BACKGROUND: Our understanding of Inflammatory bowel disease over the last ten years has increased significantly. In the past diagnostic approaches were limited to contrast radiographic studies and endoscopic examination while medical therapy centered on sulfa-derived drugs and corticosteroid therapy. The year 1998 was a landmark year in the diagnosis and management of Crohn's disease, one of the inflammatory bowel diseases. Translational research has led to useful diagnostic and therapeutic options for patients with Crohn's disease.

RECENT FINDINGS: Inflammation of the intestinal mucosa in inflammatory bowel disease is characterized by increased production of inflammatory mediators. These inflammatory mediators known as cytokines orchestrate the inflammation with the gut in Crohn's disease and ulcerative colitis. There are two classes of cytokines proinflammatory and anti-inflammatory. The pro-inflammatory cytokines include tumor necrosis factor (TNF-α), interleukin –1 (IL-1B), interleukin-2, and interferon. The production of these pro-inflammatory cytokines is increased in Crohn's Disease.

Targan and coworkers have used anti-TNF-α therapy in a series of clinical trials in Crohn's Disease that appear promising. Administration of human–mouse chimeric monoclonal antibody (Infliximab) infused at doses of 5 mg/kg has provided excellent clinical results in patients with chronically active and refractory Crohn's disease and has prolonged improvement and remissions with repeated infusions.

In addition to the pharmacological advances in IBD, a new diagnostic test developed by Rummele and Targan et al, was recently published. This assay shows promise in the pediatric group who present with nonspecific gastrointestinal complaints. It also appears useful in differentiating Crohn's disease from other gastrointestinal diseases.

Several recent reports have shown that antibodies against Saccharomyces cervisiae, (ASCA), baker's and brewer's yeast are found more commonly in the sera of adults with Crohn's disease compared to controls and patients with ulcerative colitis. Likewise, antibodies to peripheral neutrophil cytoplasmic antigen (pANCA) are typically found in patients with ulcerative colitis. The sera of patients with a broad spectrum of well-characterized gastrointestinal diseases were tested for ANCA and ASCA using enzyme immunoassays. The specificity

and predictive value of the assays in distinguishing IBD from non-IBD gastrointestinal diseases were 95 percent and 96 percent, respectively. Children with Crohn's disease typically had both IgA and IgG ASCA.

SIGNIFICANCE: Anti-TNF-α is the first drug approved by the FDA specifically for the treatment of Crohn's disease. This breakthrough was the direct result of years of clinical and basic investigation into inflammatory bowel disease that has defined the cascade of inflammatory events that occurs in Crohn's disease. From these studies, it was clear that TNF-α was a pivotal factor in the inflammatory process underlying inflammatory bowel disease. The use of anti –TNF-α, known commercially as inflixiamb, provides new hope for patients with severe Crohn's disease who have failed conventional therapy.

The expression of ASCA and p ANCA are highly specific for Crohn's disease and ulcerative colitis respectively. These new serological tests may help clinicians more accurately distinguish between Crohn's disease and ulcerative colitis; a distinction that is important in determining treatment options.

FUTURE DIRECTIONS: Other agents that act by inhibiting TNF-α or other pro-inflammatory cytokines such as IL-1, IL-6 or interferon are worthy of evaluation in animal models, and if promising, in human trials. In reference to the new serological assay for IBD, future studies are needed to determine the predictive value of using ASCA in combination with other laboratory markers in screening patients with nonspecific gastrointestinal complaints.

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Ruemmle FM, Targan SR, Levy G, Dubinsky M, Braun J, and Seidman EG: "Diagnostic Accuracy of Serological Assays in Pediatric Inflammatory Bowel Disease." Gastroenterology 1998;115:822-829.

VIII. TITLE: Helicobacter pylori and Ulcer Disease

BACKGROUND: Helicobacter pylori infection has been shown to persist for a lifetime if it is not treated and is responsible for a major morbidity and mortality worldwide. Preventative immunization may be a practical approach to the elimination of the bacterium in high-risk population groups. High rates of protection have been experimentally demonstrated in the H. felis mouse model, utilizing antigens from whole cells to purified recombinant proteins selected because of their role in pathogeneticity. In some situations, immunization is followed by resolution of the infection. At this time, urease remains the most commonly used target antigen in vaccine development studies.

Helicobacter pylori produces a urease that catalyzes the hydrolysis of urea to yield ammonia and carbonic acid. It is this urease which aids in the colonization of the host by neutralizing gastric acid and providing ammonia for protein synthesis. Host defenses are avoided by urease by continuing to neutralize acid locally and by shedding urease from the surface of the bacterium.

RECENT FINDING: Our understanding of the physiology of urease has been enhanced recently by studies conducted by the laboratory of Dr. George Sachs. In this study, the H. pylori urease system was found to be highly adaptive to an environment of varying acidity. Utilizing a microphysiometer, these investigators were able to measure bacterial metabolism and urease activity and acid or alkali resistance of Helicobacter pylori. These observations further validate the findings that urease activity is the major reason that H. pylori is an acid tolerant organism. A better understanding of how H. pylori affects the stomach as demonstrated in the Sachs study provides further support for prevention strategies such as vaccine development.

In another scientific area, several researchers demonstrate that oral immunization of H. felis infected mice with recombinant urease induces an immune response that eliminates the bacterium. Utilizing recombinant H. pylori urease antigen, Saldinger and Michetti showed both therapeutic as well as preventive effects of mucosal immunication in mice. The major responses induced by immunization were Th2 CD4 T cell responses. The elimination of H. pylori was shown to be attributable to this cellular immune response.

SIGNIFICANCE: H. pylori infection of humans has been linked to both duodenal and gastric ulcers as well as severe pre-cancerous conditions, including gastric metaplasia and atrophy. H. pylori decreases the concentration of ascorbic acid in the gastric lumen, a change that decreases the protective antioxidant mechanisms. Also ongoing observations show that H. pylori infection is

suspected to contribute to DNA damage and alteration in immune response. Saldinger and Pierre Michetti et al have shown that effective immunization with recombinant H. pylori urease generates a de novo T helper type response in infected and noninfected mice. These investigations indicate that mucosal immunization with recombiant H. pylori urease may be capable of eliminating the pathogen. These findings have major implications in the development of vaccines for humans both for the therapy and the prevention of H. pylori infections.

FUTURE DIRECTIONS: Powerful new tools such as the microphysiometer have become available and are providing new methods to understand the bacterial metabolism of H. pylori and will undoubtedly provide important strategies for new treatments and development of new vaccines. Newer techniques to understand the biology of this organism are needed to clarify mechanisms that are exhibited by different strains of H. Pylori.

In view of the success of oral immunization for prevention of H. pylori infection in mice, the next logical step is to develop and evaluate a vaccine for humans. Vaccine induced immunity may be an important means of prevention of peptic ulcer disease and gastric cancer especially in high-risk populations.

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IX. TITLE: Diarrheal Illnesses and Food Safety

BACKGROUND: Escherichia coli comprises a group of bacteria many of which are found normally in the intestines of humans and animals. Some E. coli, however, are highly pathogenic. The 0157:H7 strain of E. coli is perhaps the best known pathogenic strain. This organism was first identified in 1982 and has been designated as an emerging infection by the Centers for Disease Control and Prevention because of its increasing incidence over the last two decades.

E. coli 0157: H7 infection afflicts the very young and the very old. It produces a spectrum of illness, from mild diarrhea lasting 6 to 8 days to severe hemorrhagic colitis characterized by grossly bloody diarrhea, severe abdominal cramps, low grade fever and vomiting. Ten percent of infected individuals develop hemolytic uremic syndrome a life threatening illness characterized by acute renal failure, hemolytic anemia, and various central nervous system abnormalities.

Generally, most individuals who have E. coli 0157:H7 associated diarrhea have a complete recovery. The treatment of this illness is usually supportive with rehydyration and with blood products for severe anemia. Because of its gastrointestinal presentation, physicians may fail to consider the diagnosis, and misdiagnoses, including ischemic colitis, pseudomembranous colitis, and inflammatory bowel disease, sometimes leading to unnecessary invasive diagnostic and therapeutic procedures, or inappropriate antibiotics therapy.

RECENT FINDINGS: Strains of E. coli are characterized by their ability to produce two type of Shiga toxin. These toxins are important factors in the pathogenesis of Hemolytic Uremic Syndrome. However, the mechanisms of how these toxins cause disease are unknown. Now, a recent discovery by Bieber and Schoolnik give insight in how this strain of E. coli causes diarrheal states in humans and animals. A unique property of Enteropathogenetic E. coli is that it possesses certain features known as bundle forming pili (bfp), seen by electron microscopy. These bundle-forming pili, allow the bacteria to adhere to the lining on the intestines and confirms virulence. The type IV bundle forming pili of the enteropathogenetic E. coli have been found in in vitro studies to be responsible for promoting bacterial adherence and subsequently diarrheal states. Bundle forming pili expression is required for the development of bacterial microcolonies on tissue cultures known as localized adherence phenotype and formation of spherical bacterial aggregates in tissue culture, known as autoaggregation phenotype.

In a study conducted at Stanford University, NIDDK-supported researchers inoculated normal human volunteers with either a bfp negative or a bfp positive

(wild type) strain of E coli. Volunteers who received the wild type solution had a dose dependent diarrheal response, and those who had the bpf negative solution had less diarrhea. Thus, this study further confirms that bpf is a determinant of virulence in human diarrheal illnesses related to E. coli.

A recent study by NIDDK-supported investigators from the University of Washington provided insights into the importance of the proper diagnosis of E. coli hemorrhagic colitis and ischemic colitis. Ischemic colitis is probably the most common ischemic disorder of the gastrointestinal tract and usually presents with left lower abdominal pain, and bloody diarrhea. The specific cause for ischemic colitis is not usually identified. In a retrospective study by Tarr and coworkers, E. coli 0157: H7 bacteria were identified in a proportion of patients with presumed ischemic colitis. Utilizing immunochemical-staining techniques, the investigators identified E. coli 0157:H7 organisms in tissue sections from over one-third of cases diagnosed as having ischemic colitis. This study underscores the importance of identifying patients who present with acute bloody diarrhea and the importance of performing stool cultures for E. coli 0157:H7.

SIGNIFICIANCE: The understanding of the pathogenesis of E. coli 0157:H7 is not merely of theoretical interest but rather aids in the development of new approaches to the management of E. coli 0157 induced foodborne illnesses. The study by Bieber and Skoolnik provides insights into how E. coli colonizes and produces diarrheal illnesses in man. Their discovery that bundle-forming pili of enteropathogenetic E. coli are necessary for the development of diarrheal illnesses provides a better understanding of the role of adherence in the virulence of this organism.

The second study by Tarr et al. provide important information for clinicians. The assessment of patients who present with bloody diarrhea should include a stool culture for E. coil 0157:H7 even though colonoscopic and radiographic studies may be suggestive of ischemic colitis. The finding of the organism in tissue of patients with presumed ischemic colitis raises the possibility that the organism may have a role in initiating the event.

FUTURE DIRECTIONS: Future studies on the adherence of E. coli 0157:H7 will help elucidate the mechanism on how this organism colonizes the gastrointestinal tract and how the toxin from this organism leads to systemic disease.

In addition, there is a need for large prospective epidemiological studies of patients with bloody diarrhea to define whether E. coli 0157:H7 is a triggering event in ischemic colitis.

ACKNOWLEDGEMENTS:

Source of Support:

Grant or Contract #	Principal Investigator	Institution
RO3DK52038	Schoolnik, Gary	Stanford University
R01DK52038	Tarr, Phillip	Univ of Wash

Publication Data:

Bieber D, Ramer SW, Wu CY, Murray WJ, Tobe TT, Fernandez r. Schoolnik GK. "Type IV Pili,Transient Bacterial Aggregated, and Virulence of Enteropathogenetic Escherichia Coli." Science 1998; 280:2114-2118.

Su C, Brandt LJ, Sigal SH, Patterson K, Tarr PL. "The immunochemical Diagnosis of E. coli 0157:H7 Colitis: Possible association with Colonic Ischemia" Am J Gastroenterology 1998; 93:7:1055-1059.

GASTROINTESTINAL AIDS PROGRAM

X. TITLE: Mucosal Immunity and Aids of the Gastrointestinal Tract: Identification of the Gastrointestinal Tract as a Major Site of Viral Replication and Cd4 T Cell Depletion

BACKGROUND: The gastrointestinal tract is a major target organ in the pathogenesis of AIDS. Progressive weight loss, chronic diarrhea, intestinal malabsorption as well as the AIDS Wasting Syndrome are prominent clinical features of HIV infection. In addition, the gastrointestinal tract is the target organ for numerous opportunistic infections characteristic of late stage AIDS. Although it is well established that in acute HIV infection, the gastrointestinal tract is a portal of entry for the virus in a large percentage of cases, it is unknown what specific early events occur in the gastrointestinal mucosa that promote systemic viral infection and pathogenesis.

RECENT FINDINGS: The human and simian immunodeficiency virus (HIV and SIV, respectively) replicate optimally in activated memory CD4 T cells which are abundantly found in the gastrointestinal mucosal tissues. SIV infection of Rhesus monkeys resulted in profound and selective depletion of CD4 T cells in the intestine within days of viral infection. The depletion of T cells occurs prior to any changes in T cells in the peripheral lymphoid tissues. The loss of T cells

in the intestine is concomitant with the productive infection of mononuclear cells in the intestine.

SIGNIFICANCE: The mucosal tissues of the gastrointestinal tract comprise the gut-associated lymphoid tissue (GALT) which represents the largest reservoir of T cells in the body. The present study shows that in an animal model of human HIV infection, the GALT reservoir of T cells is the major target for virus replication. The infection of such a large number of T cells and replication of virus in these cells likely accounts for the "viral burst" or spike in viral load seen in acute human infection. In addition, this study showed a dramatic loss of infected T cells in the intestine within four days of infection indicating the initial pathogenic effect of infection with the immunodeficiency virus.

FUTURE DIRECTIONS: With the observation that the gastrointestinal tract is the major target of the immunodeficiency virus in acute infection with regard to both infection and pathogenesis, future research efforts toward a vaccine for HIV can now target infection via the gastrointestinal mucosal surfaces. A vaccine capable of preventing infection and or transmission of the virus at mucosal surfaces would prove effective at halting or decreasing the systemic infection by HIV.

ACKNOWLEDGMENTS:

Source of Support:

Grant or Contract #	Principal Investigator	Institution
R01DK50550	Lackner, A. A.	Harvard Med School

Publication Data:

Veasey RS, De Maria MA, Chalifoux LV et al. “Gastrointestinal Tract as a Major Site of Cd4 T Cell Depletion and Viral Replication in Siv Infection.” Science 1998; 280:427-31.

Veazey RS, Lackner, AA. “The Gastrointestinal Tract and the Pathogenesis of AIDS.” AIDS 1998;12A: S35-42.

NUTRITION PROGRAMS

XI. TITLE: Characterization and Expression of Intestinal Cotransporters

BACKGROUND: In recent years Dr. Ernest Wright and co-workers at the University of California cloned and sequenced the gene responsible for glucose/galactose malabsorption and accompanying water transport perturbations with severe diarrhea and dehydration. The syndrome results from a defect in the Na/glucose symporter (SGLTI), which couples glucose and galactose transport to Na gradients across the brush border membrane of cells lining the small intestine and renal proximal tubule. In addition to glucose/galactose malabsorption syndrome, other hereditary diseases believed to be due to defects in Na/glucose cotransport proteins include renal glycosuria, Hartnup disease and prolinuria. Furthermore, perturbations associated water transport result in severe health problems. For example, it is reported that worldwide, more than 10,000 children under 5-years of age die daily from dehydration. Another 3,000 suffering from dehydration are saved each day by oral rehydration therapy. This therapy is based on the fact that glucose stimulates salt and water transport across the small intestine, but basic mechanisms remain obscure. One explanation is that water transport is directly coupled to the movements of solutes by cotransporters.

RECENT FINDINGS: After cloning the gene responsible for glucose-galactose malabsorption, work has continued resulting in efficient methods for screening patients for mutations and further elucidation of mechanisms involved in defective SGLTI proteins. Studies have confirmed that each mutation causes malabsorption by reducing the number of transporters in the plasma membrane. The mutations affect the Na/sugar and water cotransport by blocking the transfer of SGLTI protein from the endoplasmic reticulum to the plasma membranes. The mutant genes possess heterozygous missense mutations and produce poorly glycosylated proteins that fail to reach surface membrane and thus do not promote glucose absorption.

Additional studies by Dr. Wright and co-workers have confirmed that water transport is directly linked to solute transport by cotransport proteins such as the brush border Na/glucose cotransporter. The Na/glucose cotransporter was expressed in Xenopus oocytes, and the changes in cell volume measured under sugar-transporting and non-transporting conditions. These investigators demonstrate that 210 water molecules are directly coupled to each sugar molecule transported and estimate that the SGLTI could account for approximately half the daily re-uptake of water from the small intestine.

SIGNIFICANCE: The current studies have led to improved methods for genetic screening of patients, with malabsorption syndromes and other related, inherited diseases. In addition, the elucidation of processes involved in cotransport of solutes and water may provide a better understanding of basic mechanisms underlying several diarrheal diseases and improved treatment of the diseases.

FUTURE DIRECTIONS: Future work should include the screening of additional patients with more direct studies of SGLTI protein processing and degradation in model expression systems to determine how missense mutations actually impair sugar and water transport. Additional studies will also be needed to establish the existence of other water/solute cotransporters and the mechanisms involved in transport perturbations in diarrheal diseases.

ACKNOWLEDGMENTS:

Source of Support:

Grant or Contract #	Principal Investigator	Institution
R01DK19567	Wright, Ernest	Univ of Calif-LA
R01DK44582	Wright, Ernest	Univ of Calif-LA

Publication Data:

Martin MG, Lostao MP, Turk E, Lam J, Kreman M, Wright EM, "Compound Missense Mutations in the Sodium-D-Glucose Cotransporter Result in Trafficking Defects," Gastroenterology 1997;112:1206-1212.

Meinild AK, Klaerke DA, Loo DDF, Wright EM, Zeuthen T. "The Human Na+-Glucose Cotransporter is a Molecular Water Pump," J Physiol 1998;508:15-21.

XII. TITLE: Assessment of Zinc Status in Human Based on Metallothionein Gene Expression

BACKGROUND: Zinc status is difficult to evaluate in humans and all methods used to date have been met with significant skepticism. Although zinc is an essential mineral with numerous physiological roles, a specific, reliable biomarker or index for zinc status has not been developed. The assessment of nutritional status usually is based on either the level of the nutrient in a blood component or on a biomarker related to a function that responds to dietary intake and/or body stores of that nutrient. Zinc status measurements are problematic because measurement of zinc levels in plasma or blood cells, while

technically straightforward, is not a reliable and sensitive predictor of zinc status. Plasma zinc concentration is homeostatically regulated at 10-15 micromole/liter. There appears to be adaptation to low zinc intake that decreases zinc excretion to achieve conservation and maintenance of of normal plasma concentration. This is achieved through several mechanisms using zinc transporters and binding proteins that control cellular influx and efflux.

RECENT FINDINGS: Recently, Dr. Robert Cousins and colleagues have demonstrated that erythrocyte metallothionein (MT) levels reflect changes in dietary zinc status in humans using an enzyme linked immunosorbant assay (ELISA). Since dietary zinc intake is directly related to cellular MT mRNA levels in rats, these investigators reasoned that an assay based on MT mRNA levels would provide a reliable measurement of zinc intake and body zinc status in humans; the MT gene is transcriptionally regulated by zinc. Using competitive reverse transcriptase-polymerase chain reaction (CRT-PCR) assays, mRNA levels for MT was found to be a reliable biomarker for zinc status during both zinc supplementation and zinc depletion. MT mRNA levels in monocytes do not show the same homeostatic responses found in plasma levels of zinc. Furthermore, actual MT protein levels in erythrocytes show similar responses to changes in zinc intake/depletion levels. Thus, both erythrocyte MT and monocyte MT mRNA should prove to be measures useful for assessment.

SIGNIFICANCE: With increasing evidence concerning the essentiality of zinc to human health, there is a need for specific, reliable indicators of zinc status and cellular responses to dietary zinc. However, several factors have made identification of such indicators difficult. Zinc metabolism is under tight homeostatic control, therefore, the most commonly used variable for measuring nutrient status such as zinc concentration in body fluids and zinc metalloenzyme activities have not been reliable for the assessment of status. Consequently, marginal zinc deficiency, which may be prevalent in several populations in the U.S. and the world, is especially difficult to detect.

FUTURE DIRECTIONS: Further studies are required to evaluate the use of the ELISA and CRT-PCR assays in dietary zinc status assessment, and the variables that may influence those measurements in various cells and tissues and under different disease states. This will be critical in further studies focusing on evaluation of dietary zinc supplements and on effects of zinc depletion in health and disease.

ACKNOWLEDGMENTS:

Source of Support:

Grant or Contract #	Principal Investigator	Institution
R01DK52412	Cousins, Robert	Univ of Florida
R37DK31127	Cousins, Robert	Univ of Florida

Publication Data:

Sullivan VK, Burnett FR, Cousins RJ, "Metallothionein Expression is Increased in Monocytes and Erythrocytes of Young Men During Zinc Supplementation," J Nutr; 1998;128:707-713.

Sullivan VK, Cousins RJ, "Competitive Reverse Transcriptase-Polymerase Chain Reaction Shows that Dietary Zinc Supplementation in Humans Increases Monocyte Metallothionein mRNA Levels," J Nutr, 1997;127:694-698.

XIII. TITLE: New Roles for Vitamin D: Effects on the Immune System

BACKGROUND: Until the 1980's, the function of vitamin D was thought to be primarily related to the maintenance of plasma calcium and phosphorus concentrations required for normal mineralization of the skeleton and for normal neuromuscular function. In more recent years the vitamin has been targeted to a number of tissues, and with the discovery of the nuclear receptor (VDR) its roles as a member of steroid-thyroid hormone super family became clearer. These earlier investigations have led to treatment of rickets, hypocalcemia of hypoparathyroidism, renal osteodystrophy, osteoporosis and psoriasis.

The VDR has been detected in cells not previously thought to be target organs, and investigators continue to discover new functions of vitamin D, most recently in control of cellular growth and differentiation. The VDR binds to response elements in the promoter region of target genes to stimulate or suppress transcription of those genes encoding for proteins that carry out a wide variety of functions, including those in the immune system.

RECENT FINDINGS: One of the most interesting sites of action of vitamin D recently described is in the immune system where the VDR has been demonstrated in activated lymphocytes. Dr. DeLuca and collaborators have found that T-cell-mediated immune responses such as delayed hypersensitivity could be inhibited by excess vitamin D or its analogs. Furthermore, a deficiency of vitamin D also interferes with the T-cell-mediated immunity. It is now clear

that vitamin D and its analogs can influence T-cell-mediated disease states in animals.

Dr. DeLuca and colleagues have used a mouse model of the human disease multiple sclerosis (MS); experimental autoimmune encephalomyelitis (EAE), a mouse disease, can be both prevented and reversed by injections or dietary ingestion of vitamin D. Furthermore, if vitamin D treatment is removed during regression, the disease process continues. Thus, the action of the vitamin D hormone can be used both to prevent the disease entirely or it can be used to arrest its development in this model of human MS. Similarly, preliminary data using vitamin D in animal models of rheumatoid arthritis and transplant rejection experiments show equally promising results. It appears that the vitamin D compounds target either the CD-8 cytotoxic lymphocytes or the Th2 helper lymphocytes that in turn suppress Th1 lymphocytes that are known to induce the inflammatory response.

SIGNIFICANCE: It appears likely that vitamin D and its analogs may be useful in modulating some immune-mediated diseases and rejection of transplants.

FUTURE DIRECTIONS: Clinical trials and studies are needed to assess effects of vitamin D and its analogs on autoimmune diseases in humans. It will be critical to ascertain effectiveness levels, concomitant effects on calcium homeostasis, and whether the compounds can be used without compromising the skeleton and without compromising the host to opportunistic infection. In addition, further basic studies are needed on mechanisms involving cytokine actions and vitamin D in the immune system.

ACKNOWLEDGMENTS:

Source of Support:

Grant or Contract #	Principal Investigator	Institution
P01DK14881	DeLuca, Hector	Univ of Wisc
R01DK46820	Hayes, Colleen	Univ of Wisc

Publication Data:

DeLuca H, Zierold C, “Mechanisms and Functions of Vitamin D,” Nutrition Reviews 1998;56:S4-S10.

Cantora MT, Woodward WD, Hayes CE, DeLuca HF, “1,25-Dihydro D3 is a

Positive Regulator for the Two Anti-Encephaletogenic Cytokines TGF- -1 and IL-4," J Immonol 1998;160:5314-9.

Hayes CE, Cantora MT, Deluca HF, "Vitamin D and Multiple Sclerosis," Pro Soc Exp Biol Med 1997;216:21-27.

OBESITY AND SATIETY PROGRAM

XIV. TITLE : Etiology and Pathogenesis of Obesity

BACKGROUND: Obesity is the most common nutritional disorder in the United States. Though commonly trivialized as primarily a cosmetic problem resulting from "lack of will power," a growing body of evidence from epidemiological, basic and clinical research indicates that obesity has a biologic basis and represents a major - and costly - threat to health. Obesity contributes to diabetes, hypertension, elevated blood lipids and certain types of cancer. Over half of all US adults are overweight (BMI >25), and more than 20 percent are obese (BMI >30). The costs of obesity to the U.S. health system are enormous. It is estimated that direct and indirect health care costs attributable to obesity approach $100 billion. Obesity is particularly common among African American and Hispanic women, with prevalence rates of more than 33 percent in these groups. Studies involving twins, adopted children and extended families suggest that as much as 80 percent of the susceptibility to obesity is due to genetic factors. The term susceptibility is used because such genes do not act in isolation, but have their effects through interactions with a host of developmental and environmental factors.

Treatments for obesity are notoriously ineffective when measured by the yardstick of success in the maintenance of reduced body weight. An understanding of the nature of the components for the set point system would provide physiologic and pharmacological tools for the successful treatment of obesity and diabetes. The advances described below result from efforts to understand the bases for body weight control at both the physiologic and the molecular levels.

RECENT FINDINGS: Interactions between Melanocortins and Leptin. Researchers at the University of Cincinnati and the University of Washington have recently identified a role of the central nervous system (CNS) melanocortin system in mediating the effects of leptin in the brain to reduce food intake and body weight. Leptin is the fat-cell derived hormone that is synthesized and secreted in direct proportion to amount of body fat. When administered either peripherally or directly into the central nervous system, leptin potently reduces

food intake and body weight in rodents. The current data indicate that CNS melanocortin system is an important target of leptin action.

The melanocortins have traditionally been implicated in the control of skin and hair color but they also have potent effects in the CNS to alter food intake. For example, several stimulators of CNS melanocortin receptors decrease food intake, while receptor blockers stimulate food intake. Importantly, leptin's ability to reduce food intake depends critically on activity at CNS melanocortin receptors. Doses of melanocortin receptor blockers that have no effect on their own can completely reverse the reduction in food intake and increased brain activity usually observed after leptin administration.

SIGNIFICANCE: Illuminating the CNS mechanisms responsible for the normal regulation of food intake and body weight provide insight into how the system may be altered in diseases characterized by energy balance dysregulation such as obesity and wasting in response to AIDS and some cancers. Understanding the signaling cascade for leptin actions in the CNS also provides other potential systems beyond the leptin receptor to target for therapeutic intervention in obesity. The current work points to the CNS melanocortin system as one important piece of that cascade and one potential point to intervene for therapeutic effect.

FUTURE DIRECTIONS: To further understand the CNS mediation of leptin actions, it will be necessary to determine how many of leptin's effects are mediated by the melanocortin system. Leptin clearly alters sympathetic nervous system activity and a variety of metabolic and endocrine factors. How many of these effects are influenced by the melanocortin system is unknown. Additionally, the functional relationship between the hypothalamic melanocortins and other CNS neurotransmitter systems important in the control of food intake is yet to be determined.

ACKNOWLEDGEMENTS:

Source of Support:

Grant or Contract #	Principal Investigator	Institution
RO1DK54080	Seeley, R. J.	Univ Cincinnati
R37DK17844	Woods, S.	Univ Cincinnati
P30DK35816	Chait, A.	Univ Wash
R01DK52989	Schwartz, M.	Univ Wash

Publications:

Seeley, RJ, Yagaloff, KA, Fisher, SL, Burn, P, Thiele, TE, van Dijk, G, Baskin, DG and Schwartz, MW (1997). "Role of Melanocortin Receptors in Leptin Effects." Nature. 390, 349.

Schwartz, MW, Seeley, RJ, Weigle, DS, Burn, P, Campfield, LA and Baskin, DG (1997). "Leptin Increases Hypothalamic Proopiomelanocoritin (Pomc) Mrna Expression in the Rostral Arcuate Nucleus." Diabetes, 46, 2119-2123.

Woods, SC, Seeley, RJ Porte, D and Schwartz, MW (1998). "Signals that Regulate Food Intake." Science, 280, 1378-1383.

RECENT FINDINGS: Development of Fat cell Hyperplasia with Obesity. Previous reports have suggested that reaching a critical fat cell size during the development of obesity may trigger the proliferation of new fat cells, however this hypothesis remained unproven. The investigators set out to test this hypothesis systematically by determining the association between fat cell size distribution, the presence of local growth factors in adipose tissue, and subsequent change in the number of fat cells in various fat depots of lean and obese Zucker rats. The obese rats had a greater percentage of large fat cells than did lean rats. The investigators found a strong correlation between the percentage of large cells in fat tissue and the release of substances from the tissue that had a greater ability to stimulation the proliferation of fat cell precursors grown in special media. They also found that this increase in proliferative activity was associated with subsequent increases in fat cell number. Therefore, this controlled study supports the hypothesis that large fat cells (which occur as obesity develops) secrete growth factors that cause more fat cell precursors to develop.

SIGNIFICANCE: This finding has implications for the prevention and treatment of obesity, because it suggests that once a certain level of obesity develops, fat cell number (as well as fat cell size) increases, making it more difficult for the very obese person to maintain weight loss over the long-term. This makes prevention of obesity more critical as a strategy for improving public health.

FUTURE DIRECTIONS: Future studies should determine the effects of dietary change on adipose tissue growth, and better characterization of local factors that stimulate fat cell growth and development. Understanding the factors that trigger the development of new fat cells may lead to the development of new strategies for the prevention and treatment of obesity.

ACKNOWLEDGEMENTS:

Source of Support:

Grant or Contract #	Principal Investigator	Institution
RO1DK47246	Martin, Roy	Univ of Georgia

Publications:

Marques, BG, Hausman, DB and Martin, RJ. "Association of Fat Cell Size and Paracrine Growth Factors in Development of Hyperplastic Obesity." Regulatory Integrative. Physiol. In press [Dec publication].

DiGirolamo, M, Fine, JB, Tagra, K and Rossmanith, R. "Qualitative Regional Differences in Adipose Tissue Growth and Cellularity in Male Wistar Rats Fedad Libitum." Am. J. Physiol. 274:R1460-R1467. 1998.

Loh, MY, Flatt, WP, Martin, RJ and Hausman, DB. "Dietary Fat Type and Level Influence Adiposity Development in Obese but Not Lean Zucker Rats." Proc. Soc. Exp. Biol. Med. 218:38-44, 1998.

RECENT FINDINGS: Role of Non-Exercise Activity Thermogenesis (NEAT) in resistance to fat in humans.

Researchers at the Mayo Clinic in Rochester, MN and Minnesota Obesity/Nutrition Research Center have investigated the observation that humans show considerable inter-individual variation in susceptibility to weight gain in response to overeating. Continued energy intake in excess of energy expenditure in necessary for the development of obesity, and understanding the physiological basis of this variation in response to overeating could improve our understanding of the factors that predispose to obesity. Changes in energy storage and expenditure were measured in 16 non-obese subjects who were fed 1000 kcal/day in excess of weight-maintenance requirements for eight weeks. Two-thirds of the increases in total daily energy expenditure was due to increased non-exercise activity thermogenesis (NEAT), which is associated with fidgeting, maintenance of posture and other physical activities of daily life. Changes in NEAT accounted for the ten-fold differences in fat storage that occurred, and directly predicted resistance to fat gain with overfeeding. Changes in basal metabolic rate and the thermic effect of food were observed but did not predict resistance to fat gain. These results suggest that as humans overeat, activation of NEAT dissipates excess energy to preserve leanness and that failure to activate NEAT may result in ready fat gain.

SIGNIFICANCE: These studies document that some lean, sedentary individuals can consume excess food and yet gain very little weight. The source of the increased energy expenditure that accomplishes this feat is not passive (e.g. uncoupling) but active. Additional energy is expended, albeit not consciously, in every day activities. If failure to activate NEAT predisposes to obesity, developing means to more readily activate activity specifically in response to overeating could help prevent obesity.

FUTURE DIRECTIONS: Little is known about the components of NEAT and their relative contribution to the changes in energy expenditure we observed in response to overeating. Additional studies will be required to fully characterize this previously unappreciated component of daily energy expenditure and to determine to what degree it is inherited and modifiable.

ACKNOWLEDGEMENTS:

Source of Support:

Grant or Contract #	Principal Investigator	Institution
R01DK45343	Jensen, M.	Mayo Clinic
P30DK50456	Levine A.	Minnesota ONRC

Publications:

Levine JA, Eberhardt NL, Jensen MD. "Role of Non-exercise Activity Thermogenesis (Neat) in Resistance to Fat Gain in Humans." Science. In press.

XV. TITLE: Pathophysiology and Treatment of Eating Disorders

BACKGROUND: Eating disorders are a serious health problem, particularly among adolescent and young adult women. It is estimated that about 3 percent of this population suffers from a diagnosable eating disorder, while many more suffer symptoms of subthreshold eating disorders. Bulimia nervosa is an eating disorder characterized by the uncontrollable urge to binge-eat usually followed by self-induced vomiting. In the majority of cases, young women voluntarily initiate these behaviors under societal pressure to maintain a slim, "attractive" figure. At some point the voluntary control ceases and patients feel "driven" to engage in binge eating and vomiting. While some progress has been made in treating disorders such as bulimia nervosa and binge eating disorder with

psychotherapy and psychotropic drugs, less research has focused on treatments based on hypothesized physiologic mechanisms that might either initiate or perpetuate disordered eating.

RECENT FINDINGS: Treatment of Bulimia Nervosa with a Serotonin Receptor Antagonist. Reseachers at the University of Minnesota have obtained physiological and clinical evidence that a biological mechanism driving (i.e. perpetuating) the bulimic symptoms involves overactivity in the afferent branches of the vagus nerve. The main functions of this bilateral nerve is to relay information from the internal organs, including the stomach, to the brain for initiation of multiple effector responses including: (1) the feeling of “fullness” and satiety in response to meal consumption; and (2) a decrease in the detection of somatic pain (pain detection threshold). Ondansetron (Zofran), a serotonin type-3 (5HT 3) receptor antagonist, was used to assess the effect of reducing vagal neurotransmission on binge eating and vomiting and on pain detection thresholds in severe (>7 bulimic episodes per week) and chronic (>2-years illness duration) bulimia nervosa patients studied under a randomized, double-blind, placebo controlled experimental design.

Double-blind treatment with ondansetron treatment (n=13) was associated with a significant reduction in binge/vomit (B/V) frequencies compared to double-blind placebo control values (n=12). Specifically, the patients randomly assigned to ondansetron displayed a 70 percent reduction in B/V frequencies from a mean of 17 episodes per week during baseline to 6 episodes per week after 4-weeks of ondansetron treatment. Two secondary indicators of disorder severity, namely the time spent engaging in bulimic behaviors and the number of normal meals consumed, provide additional characterization of the effect of ondansetron. The subjects also significantly reduced the duration of time spent binge eating/vomiting. In addition, the number of "normal" meals and snacks increased, indicating that patients were not achieving a reduction in binge/vomit frequency by restricting food intake altogether, but rather were more capable of terminating meals without having an eating episode turn into an uncontrollable binge. In addition, pain detection thresholds (PDT), previously found to be abnormal in bulimic patients also normalized in those treated with ondansetron. The re-establishment of normal PDT (and presumably normal vagal tone) by the pathological behaviors of binge eating and vomiting was also evident in a different group of bulimia nervosa patients studied in a highly controlled hospitalized setting and asked to voluntarily engage in the bulimic behaviors.

SIGNIFICANCE: Pharmacological blockade of neural transmission in the vagus nerves by ondansetron is associated with improvement in both clinical and physiological variables. Not only was ondansetron associated with a relatively rapid improvement in the primary disease symptoms of binge-eating and vomiting, but equally important, patients also displayed a return toward normal

satiety and meal patterning. Furthermore, the physiological data on PDT from untreated bulimia nervosa patients indicates the existence of a self-perpetuating, vicious cycle in which binge-eating/vomiting results in a normalization of vagal tone and conversely, not engaging in the pathological behaviors is associated with a worsening of the underlying physiology. Since binge-eating and vomiting both result in vagal nerve stimulation, it is likely that the initial voluntary acts of engaging in these behaviors result in a self-induced, oscillatory pattern of vagal hyperactivity which is no longer under cognitive control. Collectively, these findings provide one of the first indications that alterations in the function of peripheral vagal afferents can produce complex "psychiatric" symptoms and thereby suggest new classes of compounds for potential use in the treatment of this debilitating disorder.

FUTURE DIRECTIONS: Two inter-related clinical and physiological questions need to be addressed. The first involves clinical usage issues such as determining the effectiveness of ondansetron in less-severe bulimia nervosa patients; the generalizability of vagal involvement to other eating disorders involving either binge eating (binge-eating disorder) or vomiting (purging-type anorexia nervosa); and the effectiveness of using ondansetron in combination with conventional psychotherapy or anti-depressant treatments. The second experimental series involves examination of the detailed physiological mechanisms underlying the therapeutic action of ondansetron, such as the involvement of peripheral serotonin from gastric enterochromaffin in perpetuating both the binge/vomiting behaviors and the dynamic fluctuation in pain thresholds; the functional status of the peripheral 5HT 3 receptor in bulimia nervosa subjects prior to and following ondansetron treatment; and a comparison of the acute effects of ondansetron versus another 5 HT 3 antagonist with higher CNS penetrability (granisetron) on the abstinence-induced elevation in PDT and the "psychological urge" to engage in the bulimic behaviors.

ACKNOWLEDGEMENTS:

Source of Support:

Grant or Contract #	Principal Investigator	Institution
R01DK42291	Faris, P.	Univ of Minnesota

Publications:

Hartman BK, Faris PL, Kim SW, Raymond NC, Goodale RL, Meller WH and Eckert ED. "Treatment of Bulimia Nervosa with Ondansetron" (letter). Archives Gen Psychiatry 54 (10):969-970, 1997.

Faris PL, Kim SW, Meller WH, Goodale RL, Hofbauer RD, Oakman SA, Howard LA, Stevens ER, Eckert ED and Hartman BK. "Effect of Ondansetron, a 5 HT3 Receptor Antagonist, on the Dynamic Association Between Bulimic Behaviors and Pain Thresholds." Pain. In press.

Raymond NC, Eckert ED, Hamalainen M, Evanson D, Thuras PD, Hartman BK, and Faris PL. "A Preliminary Report on Pain Thresholds in Bulimia Nervosa During a Bulimic Episode." Comprehensive Psychiat. In press.

NIDDK MINORITY TRAINING AND CAREER DEVELOPMENT PROGRAMS-FY 1998

Name of Program and Description	Division	# of NIDDK Awards	NIDDK Funding Level	ORMH Collab. Funding
Minority Access to Research Careers (MARC) T-34) NIDDK Co-funds with NIGMS. Funds predoctoral faculty fellowships, visiting scientists, conferences for minority investigators and minority health issues, and honors undergraduate training in biomedical research. Summer Internship Program in the NIDDK Division of Intramural Research (students-managed by NIDDK-EEO).	DK-wide	6	$23,236	
Minority Biomedical Research Support Program (MBRS) NIDDK co-funds with NIGMS. Provides expanded opportunities for minorities to participate in biomedical research careers. Supports research projects of interest to the NIDDK at Minority and Equal Opportunity Institutions.	DK-wide	25	$1,985,728	
R-13 (Conference Grant) to the American Physiological Society, FASEB Provides support for underrepresented minority students to attend meetings of the Society, and for 36 minority high school science teachers to have summer research training in laboratories of Society members.	DK-wide	1	$74,315	

Initiatives for Underrepresented Minorities in Biomedical Research NIH-wide program initiatives to support minority undergraduate, graduate students, high school students, and faculty members on NIDDK active research grants through administrative supplements.	DK-wide	120	$4,500,000	
Research Training of Underrepresented Minorities on Institutional Training Grants (T32) Highly qualified Minority Investigators are assigned T-32 slots held in reserve for this purpose. DDEMD=5 DDDN=3 DKUHD=6	DK-wide	14	$175,174 81,437 183,000	$41,917
Pre-doctoral Fellowships (F-31) To provide support to minority students for research training leading to M.D.-Ph.D. in the biomedical sciences. DDEMD=6 DDDN=2 DKUHD=1	DK-wide	9	$132,269	
Cell/Molecular Biology Student/Teacher Learning Center (R-25) Laboratory Research experience for minorities in the District of Columbia (managed by NIDDK-EEO).	DK-wide	1	$334,767	

<u>Small Research Grants (R-03) for Minority Researchers</u> DDEMD=5 DDDN=n/a DKUHD=1 ORMH Collaboration provides additional support for minority researchers.	 DK-wide ORMH	 6	 $367,229 84,750	 $466,933
<u>Minority High School Student Summer Research Training Supplement</u> In conjunction with the National Minority Organ Tissue Transplant Program award to Howard University, NIDDK provides meaningful laboratory research experience to minority high school students to stimulate their interest in careers in biomedical science.	 DK-wide	 1	 $70,138	
Totals		183	$8,012,043	$508,850